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Growth of the eye lens: I. Weight accumulation in multiple species

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Purpose: To examine the accumulation of wet and/or dry weight in the ocular lens as a function of age in different species.
Methods: Wet weights and/or fixed dry weights were obtained from measurements in the author's laboratory and from the literature for over 14,000 lenses of known-ages, representing 130 different species. Various algorithms were tested to determine the most suitable for describing the relationship between lens weight and age.
Results: For 126 of the species examined, lens growth is continuous throughout life but asymptotic and can be reasonably described with a single logistic equation, $W=W_m e^{-(k/A)}$, where W is lens wet or dry weight; W_m is the maximum asymptotic weight, k is the logistic growth constant and A is the time from conception. For humans, elephants, hippopotami, minks, wild goats and woodchucks, lens growth appears to be biphasic. No gender differences could be detected in the lens weights for 70 species but male lenses are reportedly 10% larger than those of females in northern fur seals and pheasants. Dry weight accumulation is faster than that for wet weight in all species except birds and reptiles where the rates are the same. Low lens growth rates are associated with small animals with short gestation periods and short life spans.
Conclusions: Lens growth is continuous throughout life and, for most species, is independent of gender. For most, growth takes place through a monophasic asymptotic mode and is unaffected by events such as hibernation. This makes lens weight measurement a reliable tool for age determination of species culled in the wild. Compaction of the growing lens generates different properties, appropriate to an animal's lifestyle. How these events are controlled remains to be established.

Vertebrate eye lens growth occurs through a unique and ubiquitous mechanism [1]. New epithelial cells produced in the proliferative zone, anterior to the equatorial region and just inside the capsule, migrate through the equatorial zone and differentiate into fiber cells [2]. The new fiber cells are laid down over existing cells to form a layered structure, resembling that of an onion. Cellular organelles are lost during this process and, consequently, so are most metabolic activities, including the ability to synthesize and break down proteins. Water may also be lost from the fiber cells, leading to increases in protein concentration, refractive index and lens power. Changes in the shape of the lens may augment or diminish the increase in power. These processes appear to continue throughout life. Since no cells or their contents, other than water and organelles, are lost, the lens retains a record of its growth and its properties continually change. Much can be learnt by studying the properties of the lens at different ages.

Little is known about the actual growth of the lens in different species and the processes whereby lenses with different properties, appropriate for the specific visual requirements of an animal, are generated. Lenses from birds are very soft and pliable while those from rodents are like rocks. Most mammalian lenses lie in between but there are differences between the nucleus and cortex. The softer lenses can be deformed, altering their focal lengths to allow accommodation whereas the hard lenses appear to be designed for specific fixed optical functions. Lens shape varies from near spherical to ellipsoid [1] and the refractive index may be graduated or uniform, providing a wide range of optical capabilities [3].

There does not appear to be consensus among those studying the lens regarding the growth characteristics and properties of lenses from different species. For example, opinions differ on whether there are gender differences or whether there is any growth at all in adult life [1,4,5]. Several algorithms have been developed to describe the relationship between lens growth and age but most of these suffer from a failure to recognize that lens growth commences during gestation, not at birth. As discussed previously, this has led to invalid conclusions about the effects of environment and nutrition on lens growth [1].

The present report has arisen from a long-term program aimed at documenting the growth of the lens in different

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species and identifying factors which may be important in directing this growth. From a combination of measurements made in the author's laboratory and those available in the literature, it has been possible to assemble detailed data on dry weight accumulation in 121 species and wet weight accumulation in 39, as well as estimates of maximum lens weight for another 17 and 20, respectively. This is a unique body of data which would be unlikely to ever be assembled again. It is presented in the hope that others may find it useful, add more data and undertake further analyses which may provide insights into the factors important for lens growth.

METHODS

No animals were sacrificed for the purposes of this study. All fresh lenses were obtained from animals which had been sacrificed for other purposes such as food production, official government culls, or other research projects and from mortalities at zoological facilities. In some cases, data sets were accumulated from occasional samples obtained over a period in excess of 40 years.

Fresh eyes from animals of known-ages were collected for 59 species. When eyes were received within two hours of death, the lenses were removed, weighed immediately and then stored on ice until further processed. For some species, lens protein contents were determined as described previously [6]. For the others and for the eyes received more than 2 h after death, lens dry weights were determined after fixing the lenses or whole eyes in 5% buffered formalin for a minimum of 2 weeks, followed by drying of the isolated lens at 80 °C until constant weight was achieved. Drying generally took 10–14 days.

Wet and/or dry weight data for 98 species were extracted from published studies which included information on the relationship between lens weight and age. Where the data were available only in graphical form, plots were scanned, magnified at least 10 fold and printed before the positions of points were measured to the nearest 0.5 mm with a ruler or to the nearest 0.1 mm using a computer's MS Word ruler (Redmond, WA). The coordinates were then converted to ages and weights by reference to measurements from the axes. It was estimated that data obtained in this way were accurate to within 2%. Information on the gestational period, maximum possible life span and maximum normal bodyweight were obtained from a variety of sources, including the listings by Altman and Ditmer [7] and Grzimek [8].

The data were analyzed using a variety of growth algorithms, most of which are available in the program Growth II (Pisces Conservation Ltd, Lymington, UK) as well as the 2 parameter logistic equation described previously [1,6,9] and by regression of age on lens weight. Since lens growth commences early in gestation, all ages used in the analyses are since conception. Regression analysis of logistic plots was used to determine the slope, which corresponds to the growth constant (k), and the y-axis intercept, which is used to calculate the maximum, asymptotic weight (W_m).

Data for related species were grouped and colors were assigned to the groups – bats (black), birds (light blue), carnivores (red), ectothermal species (reptiles, amphibians, fish, yellow with black outline), lagomorphs (purple), marsupials (dark green), primates (light green), rodents (orange with black outline), tree shrew. (light green with black outline) and ungulates (dark blue).

RESULTS AND DISCUSSION

Age-related changes in the weight of the lens (wet, or dry or both) were obtained, from measurements in the author's laboratory and from the literature, for a total of 130 species. The number of different aged lenses examined generally varied from 10 to 1200, with most in the range 100–200. For a small number of species obtained from the literature, there were fewer than 10 data points but each of these was derived from large sets of lenses. Information on the species, including the scientific (binomial) name, gestational period, estimated maximum possible life span and maximum normal bodyweight are presented in the Appendix 1, together with the number of lenses used in the analyses, references for the source of the data [10-155] and the results of the logistic analyses described below. Closely related species are grouped and color coded as indicated earlier. These colors will be used consistently throughout all data presentations. The tree shrew has been placed with the primates even though it is now not considered to be a primate and assigned to a separate order (Scandentia**)** but still within the same clade **(**Euarchonta). However, it has not been included in any analyses of the primates.

It should be noted at the outset that some of the data sets obtained from the literature may not be strictly comparable. This could be especially true for dry weights since drying and fixing conditions employed in different laboratories were highly varied. Fixation of eyes ranged from 2 days to 9 months in 5%–10% formalin and isolated lens drying ranged from 24 h at 37 °C to 24 h at 100 °C and from 1 to 20 days at 80 °C. In addition, many of the ages cited were not accurately known but rather, were estimated from consideration of changes in other body parameters such as molar progression, cementum ring counts, laminary indices, limb dimensions, the stage of epiphyseal cartilage ossification, fish scale morphology, etc. It has not been possible, in many

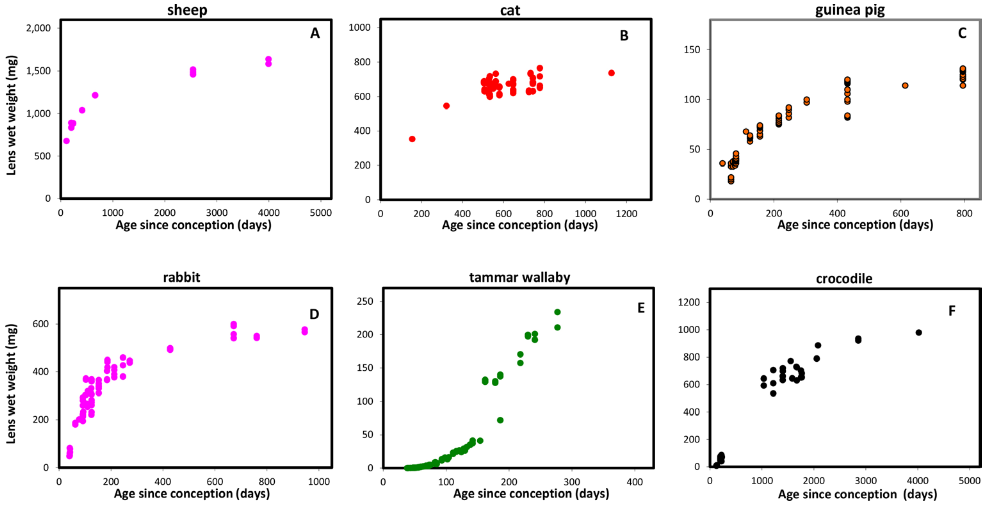

Figure 1. Changes in lens wet weight as a function of age. Weights are shown for (**A**) sheep (1,200 lenses or data), (**B**) cats (44), (**C**) guinea pigs (66), (**D**) rabbits (710), (**E**) Tammar wallabies (137), and (**F**) crocodiles (91). Color coding and the origins of the data are as indicated in the Methods section and Appendix 1.

instances, to establish how accurate these estimates might be. For example, growth rings on elephant teeth are reported to be difficult to measure, leading to age underestimates in older animals [10,11]. For 9 fish species obtained from or near the Sea of Oman [12], reservations about the reliability of the data presented precluded their inclusion in the present study.

Wet weight accumulation: Changes in lens wet weight over a substantial part of the life span were available for 39 species. Lens wet weights measured in the author's laboratory ranged from a low of 0.2 mg for a newborn dunnart to 7,500 mg for an adult bluefin trevally. Six examples of the growth curves obtained are shown in Figure 1. These were selected to demonstrate the range in quantity and quality of the available data.

Although there is scatter in some of the plots, it is clear from these figures that wet weight accumulation, i.e., lens growth, is continuous throughout life in all species, rapid early in life and gradually slowing toward an apparent asymptotic maximum. Similar curves were obtained for the other 33 species.

Dry weight accumulation: Data on the relationship between lens dry weight and age were obtained for 121 species. Measured dry weights ranged from a low of 0.15 mg for a newborn mouse lens to over 5,000 mg for the bluefin trevally. Examples of the dry weight growth curves are presented in Figure 2.

As with the wet weights, the data indicate growth is continuous throughout life and slows toward an apparent asymptotic maximum. Similar curves were obtained for all but 6 of the data sets.

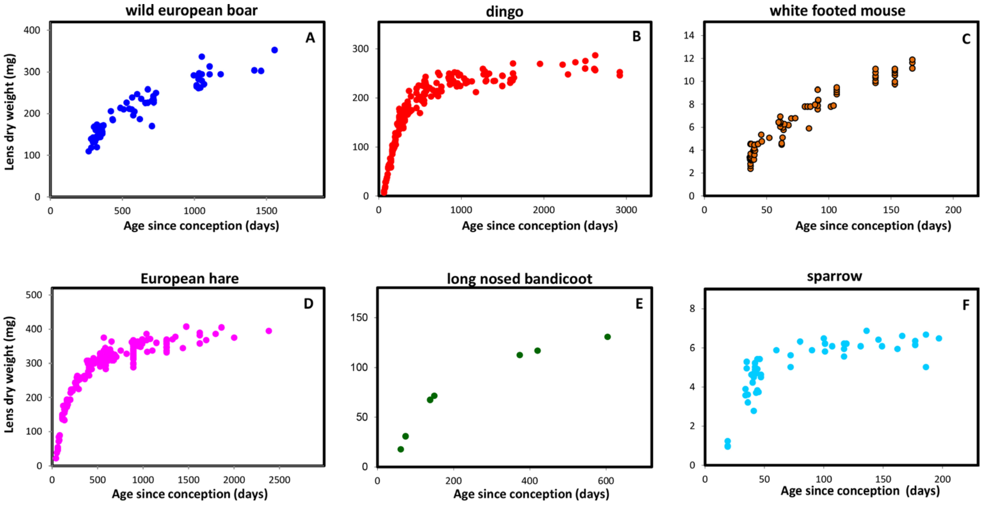

Figure 2. Changes in lens dry weight as a function of age. Weights are shown for (**A**) wild boar (113 lenses or data), (**B**) dingoes (185), (**C**) white-footed field mice (69), (**D**) European hares (88), (**E**) long-nosed bandicoots (7), and (**F**) sparrows (50). Data sources are as indicated in Appendix 1.

Growth analysis: For both wet and dry weights, differences are seen in the shapes of the lens weight versus age curves (Figure 1 and Figure 2) suggesting that the growth rates may vary in different species. Thus, the dry weight appears to increase more rapidly for the dingo than it does for the wild boar and others. However, the apparent shape of the plots is dependent on the time frame for which data were available, relative to the life expectancy. The wild boar data represent only 15% of the animal's possible life span while those for the dingo encompass over 60%. A more reliable assessment of the growth rates requires fitting of growth algorithms to the data.

To identify the most appropriate way to describe lens growth, several growth functions, including various versions of the logistic, Bertalanffy, Gompertz, Janoschek and Richards relationships were tested. The following version of the two parameter logistic-type equation, used previously for analysis of kangaroo and rabbit lens growth [6,9], was selected because of its simplicity and because it yielded best fits of the data for most species.

$$W = W_m * e^{-(k/(Ap+1/c)}$$

Where W is lens weight, W_m is the maximum asymptotic weight, A_p is the postnatal age, k is the logistic slope and c is an age constant. This equation is very similar to that first used by Lord [13]. In studies where the Lord relationship was used, 1/c was allowed to vary to obtain the best statistical fit of the data [13-17]. However, as discussed previously [9], the term 1/c represents the prenatal time from lens formation to birth and cannot be greater than the gestational period. It is appreciated that lens growth does not commence until well after conception, but the precise time is not known for most of the species examined in the present study. Therefore, the gestational period (G) was used for 1/c, so that the total age ($A=A_p+G$) of a lens corresponds to the time since conception. For the 12 species where the time of lens placode formation was known, regression analysis indicated that the age since conception generally gave a slightly better fit of the data than the time since placode formation. Thus, Equation 1 reduces to

$$W = W_m * e^{-(k/(A))}$$

Logistic analysis: Data were plotted according to the transformed version of the two parameter logistic equation

$$\ln(W) = \ln(W_m) - (k / A)$$

The logistic plots for the wet weights shown in Figure 1 are presented in Figure 3 while the dry weight plots, corresponding to Figure 2 data, are shown in Figure 4. Each of the data sets shown yields a single straight line for the whole of the available time frame, indicative of self-limiting monophasic growth toward an asymptote. With the exception of those discussed below, all other species also yielded reasonable to good linear logistic plots (R^2, 0.60–0.99) but substantial differences were evident, in both the slopes and intercepts.

The logistic slope, k, and the maximum asymptotic weight, W_m (=exp$^{\text{(y-axis intercept)}}$), were determined from these plots. They are presented in The Appendix 1. For species with short gestation times, using the time since lens placode formation, rather than the gestation period, generated plots with lower slopes but this had little effect on W_m. The slopes and intercepts of species with long gestation periods were unaffected.

Possible exceptions: Red squirrel data [18], on first analysis, yielded a curved logistic plot. However, it was found that the

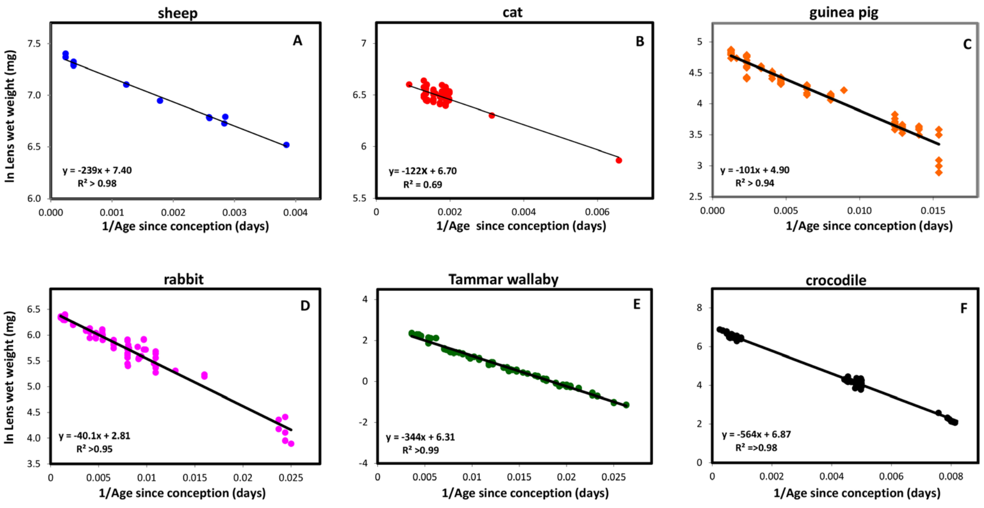

Figure 3. Logistic analysis of the changes in lens wet weight as a function of age. Data shown are for the species shown in Figure 1: sheep (**A**), cats (**B**), guinea pigs (**C**), rabbits (**D**), Tammar wallabies (**E**), and crocodiles (**F**). Data sources are as indicated in Appendix 1.

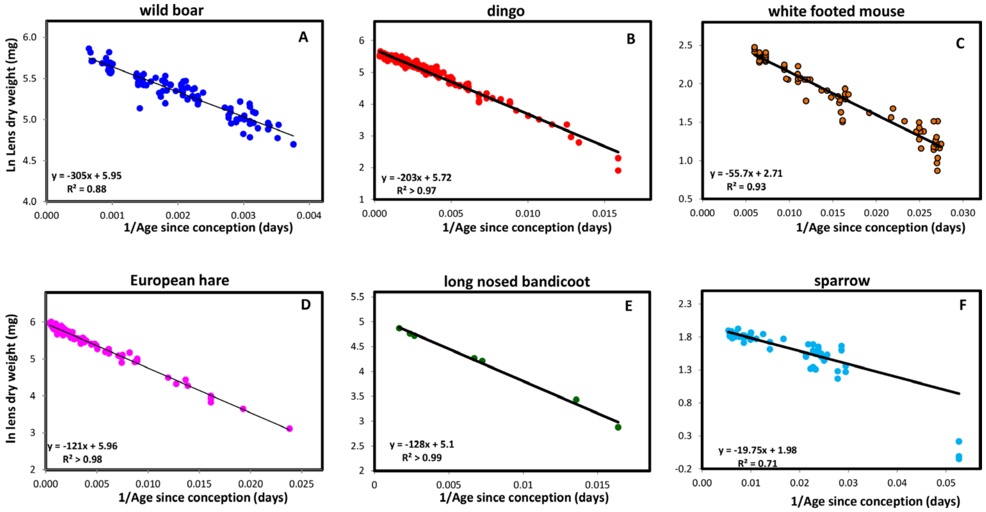

Figure 4. Logistic analysis of the changes in lens dry weight as a function of age. Data are for the species shown in Figure 2: wild boar (**A**), dingoes (**B**), white-footed field mice (**C**), European hares (**D**), long-nosed bandicoot (**E**), and sparrows (**F**).

authors had estimated ages for the 4 oldest animals based on the assumption that lens growth was linear. After elimination of these points, leaving only the actually known-age data, a monophasic growth plot (single straight line) was obtained.

Initial analysis of the smooth dogfish data also yielded a curved plot. It was noted that the ages cited in the report [19] were estimated from body lengths by comparison with those of the spiny dogfish since no other information was available at the time. However, the growth characteristics and life cycles of these two species are quite different. Recently obtained body length/age data for the smooth dogfish [20] have indicated that the previous age estimates were almost double the true ages. Adjustment of the ages generated a single straight line for the logistic plot.

Six of the data sets did not yield single straight lines on logistic analysis. For one of these, human, it has previously been shown that the lens grows in a biphasic manner [5]. The other exceptions, all from the literature, were the African elephant [10,11], American mink [21], hippopotamus [22], Spanish ibex [23] and woodchuck [24,25]. The growth curves and corresponding logistic plots for two examples, the elephant and the woodchuck, are presented in Figure 5.

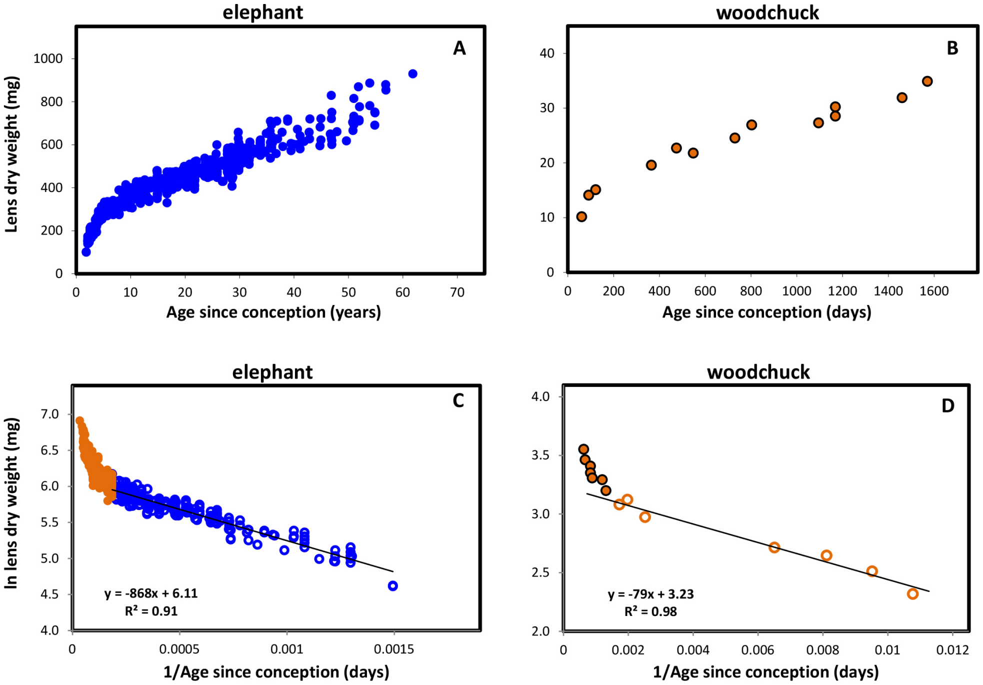

Figure 5. Species exhibiting non-asymptotic growth. Growth curves (**A**, **B**) and logistic transformations (**C**, **D**) of lens weights from the elephant (**A**, **C**) and the woodchuck (**B**, **D**).

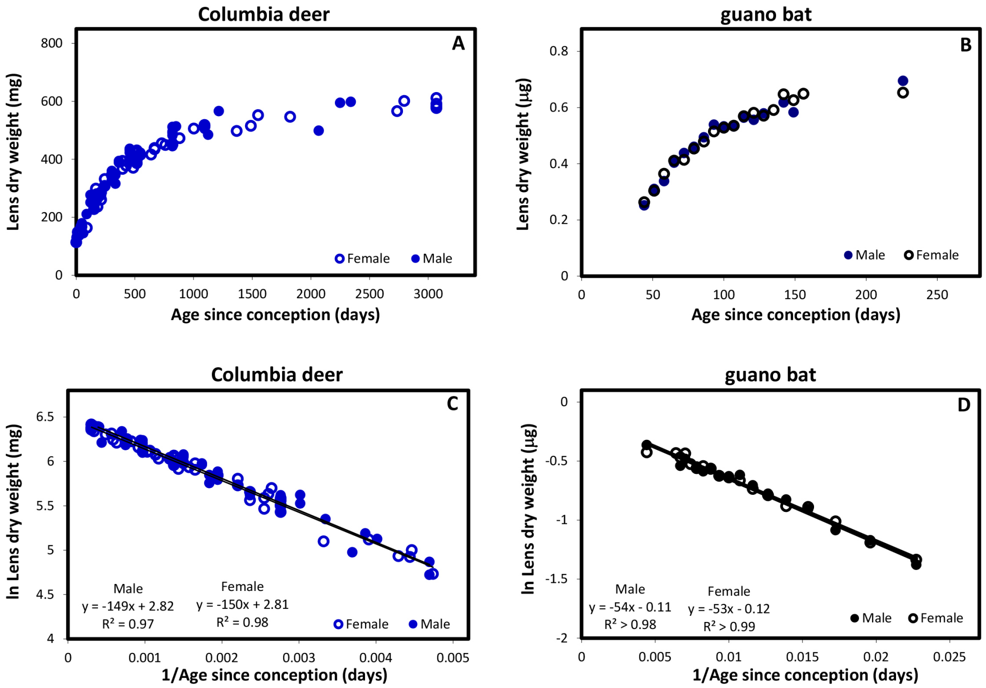

Figure 6. The influence of gender on lens growth. Growth curves (**A**, **B**) and logistic analyses (**C**, **D**) are shown for the guano bat (**A**, **C**) and the Columbian black-tailed deer (**B**, **D**).

The logistic plots show distinct upward curvatures at low 1/A (i.e., high ages), consistent with a change in growth mode. Curve fitting indicated that both sets of data can be described with an asymptotic growth phase early in life followed by linear growth, as has been observed for the human lens. A similar conclusion was made for the hippopotamus. The transitions seem to occur around 6–8 years for the elephant, 3–4 years for the hippopotamus and 500–600 days for the woodchuck, respectively. Insufficient data are available to permit more precise estimations. The slopes of the linear phases for the elephant, hippopotamus, human and woodchuck were estimated to be 10.5, 5.3, 0.54, 0.44 years, respectively.

The American mink [21] and Spanish ibex [23] data also yielded biphasic logistic plots. However, the weights of young lenses were very high, relative to the adult lenses. Young male mink lenses were reported to be over 30% heavier than those from females of the same age. In view of observations on 71 other species (see below), this is unlikely to be correct. No adult gender data were presented. Lens weights for young ibexes also appeared to be high but this might be due to inaccurate age estimates. Adult lens data only were used for analyses of these two species.

Some of the other data sets showed a small amount of upward curvature in the dry weight logistic plots at low 1/A values. This curvature was not considered significant as it was generally associated with inadequate drying procedures, such as 24 h at 55 °C. Since the time required for lens drying varies with lens size as well as temperature [9], the larger older lenses may not have dried completely when mild drying conditions were used. For example, it was reported that giraffe lenses had not reached constant dry weight after 120 h at 80 °C [26].

Gender: The gender of the lens donors was known for 73 sets of data, permitting an assessment of possible gender differences. Two examples, the guano bat [27] and the black tailed Columbian deer [28,29] are presented in Figure 6. It is clear that lens weights in males and females of the same age are indistinguishable. The same conclusion was reached for a total of 66 species.

For the remaining seven species (American mink [21], beagle dog [30], corn mouse [31], European common vole [32-34], northern fur seal [35], pheasant [36,37] and Wistar rat [38]) male and female lenses have been reported to differ. However, it is probable that some of the apparent differences are not real. As mentioned earlier, there appear to be problems with the young American mink data. With the beagle dog [30], it was not really possible to make any definitive conclusion since the data were highly scattered and there was also substantial overlap between the genders. By comparison, no suggestion of a difference was observed with the closely related dingo or with other carnivores. Male Wistar rat lenses were reported to be larger than those from females in one study [38] but this was not observed in another [39] or in the author's laboratory. Furthermore, several other species of rats show no difference between males and females.

Janova and colleagues concluded that lens dry weight is not reliable for estimating age in the vole, *Microtus arvalis*, because lens growth is different between males and females and between reproducing and non-reproducing females [34,40]. However, such differences have not been observed by others in *M arvalis* [32,33] or in other voles. Although male and female corn mice, older than 11 weeks, were reported to differ [31], the comparison was based on the average weights for 4 broad age groups. It was not possible to assess whether the age distributions within the male and female groups were comparable. Lens weights have been found to be the same in numerous other small rodents. It is concluded that male and female lenses in beagle dogs, corn mice and Wistar rats are probably not different and, therefore, the male and female data were combined for further analyses.

Insufficient information is available to assess the reported gender differences in the pheasant and fur seal. The separate male and female analyses for these are included in Appendix I. The logistic slopes (growth rates) for the genders are the same but the maximum asymptotic weights differ. The female weights were used in subsequent data analyses.

Thus, for 71 out of 73 species examined, there appear to be no differences between male and female lenses. There is no a priori reason for male and female lenses to be the same or different but, intuitively, one would expect them to be the same since lens function would be unrelated to gender. If, like many other organs, lens size scales with body size, and since male body size is frequently greater than that of females, it might be expected that male lenses would also be larger. However, male body size is not always larger, sometimes females are larger, and for many species, there is no difference or the difference is subtle and does not manifest until sexual maturation. By then, the lens has generally reached 80% of its maximum asymptotic value and subsequent differences in lens growth would have no obvious benefit to either gender. On the basis of these considerations, it is concluded that for most, if not all species, there is no difference between male and female lens weights.

The growth constant: The rate at which lens weight approaches the asymptotic maximum, i.e., the growth constant, is indicated by the slope of the logistic plot. For warm-blooded animals, exhibiting monophasic growth, the slopes range from 20 to 600 days for dry weights and from 27 to 485 days for wet weights. Where data are available for both wet and dry weights, logistic slopes are usually 10%–20% higher for dry weights. The only exceptions are crocodile and chicken where the slopes are identical. Very high slopes are found with the ectothermal species, fish, amphibians and reptiles (The Appendix 1). Insufficient data are available to permit meaningful comparisons of these since some are ovuliparous (ova fertilized externally) and others are viviparous (young develop in mother). In addition, animal growth rate and gestational time (as well as gender of the newborn) are dependent on the temperature of the external environment. Therefore, only warm blooded animal data will be analyzed and compared in the following discussions but reliable ectothermal animal data will still be presented.

Within groups of closely related species, the logistic slopes are generally similar, with the lowest slopes found in the birds and the highest among the ungulates (The Appendix 1). Nevertheless, some members in the groups differ, suggesting that the slopes may reflect some parameter other than taxonomic relationship. To determine what parameters may contribute, the slopes were compared with several other properties using allometric analyses. This included the asymptotic maximum lens size, normal maximum bodyweight, maximum possible life span and gestation period. The comparisons for lens dry weight in warm-blooded animals with monophasic lens growth are presented in Figure 7. Similar relationships were observed with the wet weights, but fewer data were available.

Low slopes (30–100 days) are associated with small lenses, low bodyweights, short life spans and short gestation periods. The slope increases as each of these parameters increases, not only between the taxonomic groups but also within them (Figure 7A-D). In all species, the lens reached 50% of its maximum dry weight at around 3 times the length of the gestation period, corresponding to around 5% of the maximum life span. At the time of sexual maturation, dry weight was around 80% of the maximum.

It might have been expected that the closest relationship of the growth constant would be observed with gestational time since it can be shown, by rearrangement of Equation 1, that the logistic slope is related to the term describing the gestational lens growth period (1/c). However, primates have lower slopes and marsupials, higher slopes than other animals with the same gestational times. For the 83 species remaining after excluding the marsupials and primates, the relationship was

$$k = 7 * G^{0.75} (R^2 = 0.89)$$

Bodyweight (BW) also yielded a good fit with birds and marsupials appearing to differ from the others. Excluding these two groups,

$$k = 25 * BW^{0.22} (R^2 = 0.87)$$

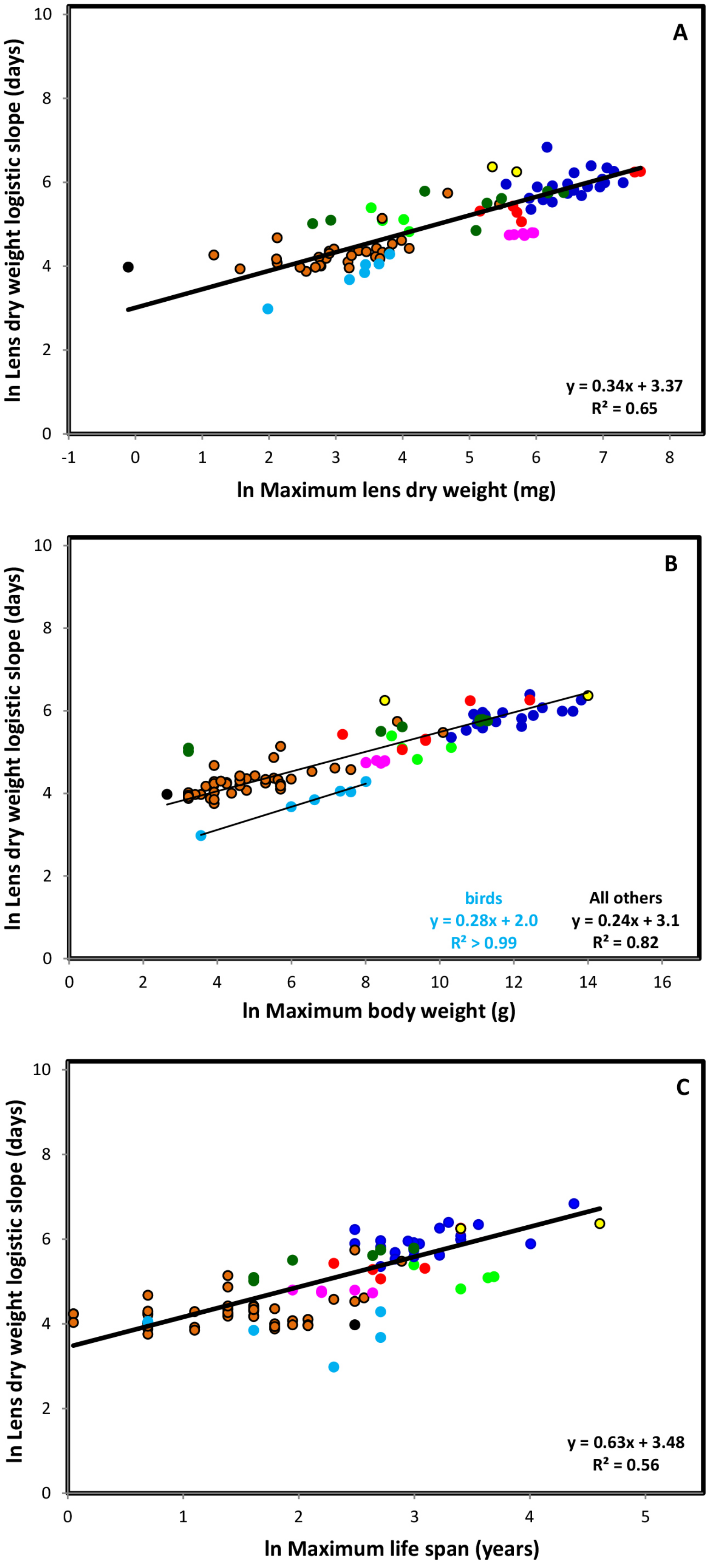

Figure 7. Allometric analysis of the relationship of the lens growth constant to different parameters. Comparisons were made with maximum lens dry weight (**A**), maximum possible life span (**B**), normal maximum bodyweight (**C**), and gestational period (**D**) in warm-blooded species. Data for the species that appear to exhibit biphasic growth have been omitted. Data for related species are shown in the same colors: bats (black), birds (light blue), carnivores (red), lagomorphs (purple), marsupials (dark green), primates (light green), rodents (orange with black outline), tree shrew (light green with black outline), and ungulates (dark blue).

Although quite good fits of the data were obtained for each of the analyses, especially when apparently outlying groups were excluded, it is concluded that parameters, other than those tested here, are responsible for regulating the rate of lens growth. However, it is still possible that the rate is determined by bodyweight or gestation time and that the species which do not fit the general trend represent adaptations for a specific lifestyle.

Five of the species examined- the chipmunk, garden dormouse, ground squirrel, hamster and woodchuck - undergo ‘true’ hibernation (lowered body temperature with low metabolic, breathing and heart rates) in winter. None of these exhibit variations in lens growth rate which can be attributed to the periods of hibernation. As mentioned above, there appears to be a transition in woodchuck lens growth at around 1.5 years but this is well past the time of the first hibernation. Thus, it would appear that lens growth continues unabated during hibernation.

Age determination: The continuous growth of the lens throughout life offers the possibility of using lens weight for determining animal ages. This was realized by Lord [13] who applied the method to cotton tail rabbits and derived an algorithm for the rabbit which is essentially identical to the logistic type equation used here. The only difference is in the age term. Lord used (postnatal Age + c) where c is a constant determined from the best fit of the data. This should correspond to the time of prenatal lens growth. Several authors have since collected data on lens weights to develop relationships for estimating ages for animals culled in the wild. Many of these used the Lord approach. Unfortunately, because of the variability in data collected in the wild and imprecise estimates of age, the best fit approach frequently yields values of c, well in excess of the gestation period. As discussed previously [9], this can lead to erroneous conclusions regarding factors affecting lens growth. The data from the published studies have been used in the current analyses but were reinterpreted using the gestation time instead of the Lord age constant (c).

Several other approaches have been used for analyzing lens growth data. A frequently used one is to regress known postnatal age on lens weight and fit a linear function. This has been used for predicting ages. As can be seen in Figure 8, a good linear fit was observed for the black rat data using this approach (R^2=0.90) [41]. (Note that $\log_{10}$ is used for this regression, as in the data source [41] rather than the ln used in the present study.) However, for the rabbit [9] the relationship is clearly sigmoidal. Re-analysis of the rat data indicates that a sigmoidal fit is slightly better (R^2=0.93) than the linear. The apparent linearity of the relationships, also reported for several other species [42-47], reflects the limited age ranges examined. Samples from early and late in life are required to reveal the sigmoidal relationship. Use of this apparently linear relationship for determining ages will result in overestimates for the old animals and underestimates for the young. A better fit of the data are obtained if age since conception is used for the regression.

Several attempts have been made to use lens wet weight or fixed wet weight (but not dried) for age determination. This is unsatisfactory since lens hydration, and hence the weight, varies with post-mortem and/or fixation time. Fixed dried lens weight is best but care must be taken that drying is complete. It was noted, during compilation of the data for this study, that, in studies where short drying times at low temperatures were used, the data were variable, especially for large lenses.

It is concluded that the logistic type equation used in the present study provides a satisfactory algorithm for age determination using dried lenses. As described previously, lenses must be fixed for at least 2 weeks and dried at >80 °C until the weight is constant, a process which will take over 2 weeks for large lenses.

Compaction: Both wet and dry weights were available for 32 species, permitting an assessment of the change in the average concentration of lens solids with increasing age and the maximum concentration reached, i.e., the amount of compaction possible in different species. Limited adult data were available for another 9 species.

The logistic plots for wet and dry weight accumulation in the chicken, Norwegian rat and sheep are shown in Figure 9. These reveal interesting differences.

For the rat and sheep (Figure 9A,B) the logistic slopes are different indicating that wet and dry weights accumulate at different rates, with the dry weight accumulation being the more rapid. This is also evident in the plots showing the ratios of dry weight/wet weight (Figure 9D,E). With increasing age, this ratio increases toward the asymptotic maximum which can be calculated from the logistic intercepts, 0.40 for the sheep and 0.45 for the rat. These ratios correspond to maximum dry weight concentrations of around 45 and 50% (w/v) respectively. Similar increases were observed for most of the other species.

By contrast, the logistic plot slopes for the dry and wet weights in the chicken are the same (Figure 9C), indicating that wet and dry weights increase at the same rate. Consequently, as can be seen from Figure 9F, the dry weight/wet weight ratio remains constant at near 0.27 throughout life. The crocodile was similar but with a lower constant ratio of

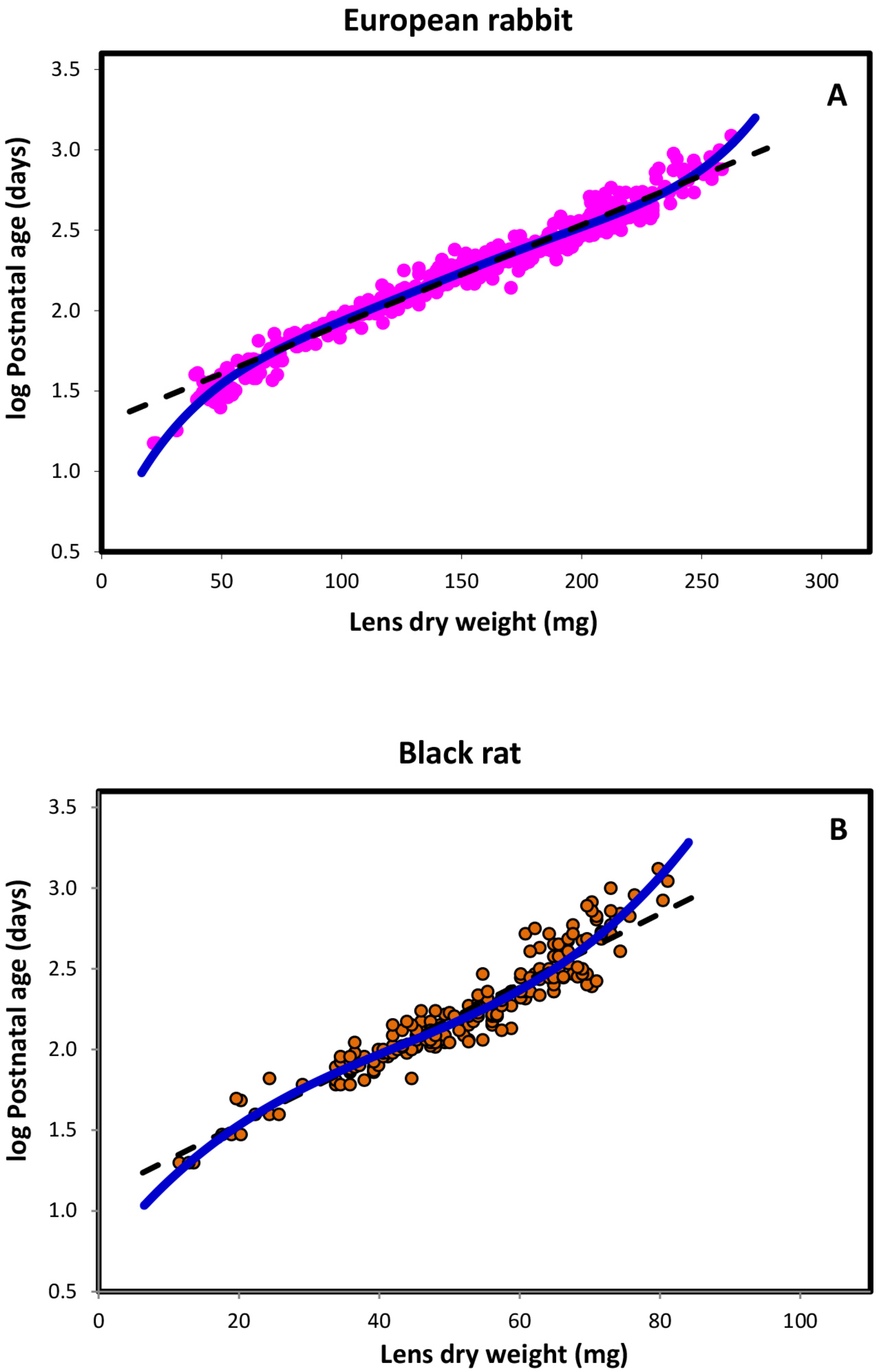

Figure 8. Regression of age on lens weight. Calculations for the European rabbit (**A**) and black rat (**B**) are presented. The sigmoidal (solid line) and linear (dashed line) fits are shown.

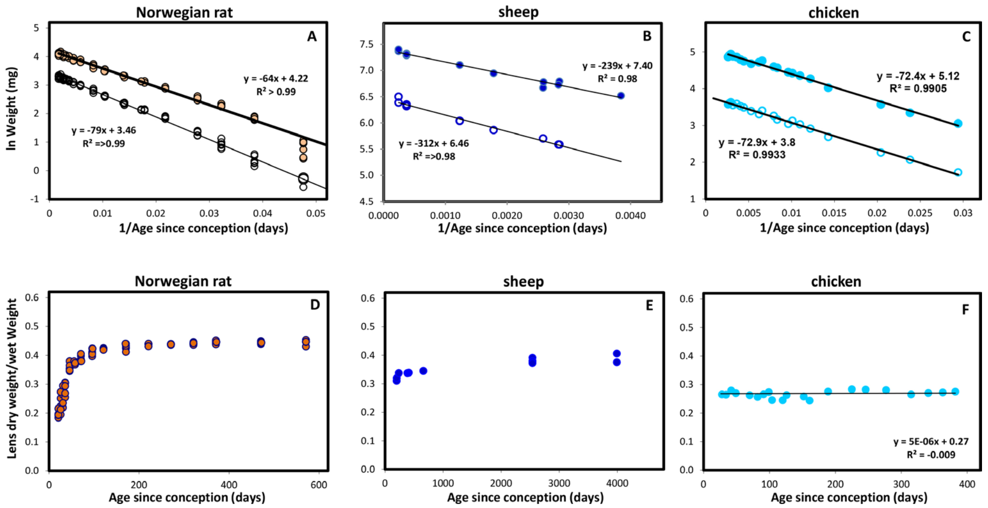

Figure 9. Lens compaction with age. Logistic analyses of wet and dry weights (**A**, **B**, **C**) and the dry/wet weight ratios as a function of age (**D**, **E**, **F**) are shown for rat (**A**, **D**), sheep (**B**, **E**), and chicken (**C**, **F**) lenses.

around 0.20. These observations indicate there is no compaction with age in the chicken and crocodile lens and, hence, probably no refractive index gradient. Low ratios were also observed with adult lenses from the little penguin (0.26), mallard (0.27) and Jackson's chameleon (0.25). However, the numbers of lenses available were too small to determine if these ratios are constant with age. The low ratios of the chicken, little penguin and mallard lenses are consistent with the conclusion that these have monofocal optical systems [48] while the higher ratio of the boobook owl lens (0.31) is consistent with its multifocal system [48].

The very low concentration of solids in these lenses indicates that they are very soft and flexible, as was been observed during processing, and capable of large accommodative changes. This would be especially so in the crocodile lens, probably reflecting the need for large lens shape changes when vision switches between air and water.

The asymptotic ratios of dry weight/wet weight and the calculated densities, for the species where data were available, are presented in Appendix 2, together with values calculated from adult lenses where lens numbers were low. The ratios range from 0.2 in the crocodile to >0.6 for several rodents and the corresponding densities from 1.05 to 1.22. It should be noted that these are average values for the whole lens. In most lenses the dry mass is distributed along a concentration gradient with the highest concentration in the center. This generates the refractive index gradient necessary for reducing eye length. The density in the center would be considerably higher than the average.

Allometric analysis (log Dry weight versus log Wet weight) also shows that dry weight increases more rapidly than wet weight in most species, indicative of compaction. Examples are included in Figure 9G,H,I for the chicken, rat and sheep. The allometric compaction constants determined from the slopes of the allometric plot range from 1.00, in birds and reptiles, to 1.33 in mammals.

Concluding remarks: The availability of the above detailed information on lens growth in so many species makes it possible to make several conclusions. Lens growth in most species is monophasic, continuous throughout life, slowing toward an asymptotic maximum. For species with low body-weights and short gestation periods, growth in adults is so slow as to appear to have stopped. This slow growth makes it difficult to use lens size as a criterion for determining age in older animals, especially in small-bodied species. However, determining annual growth classes from dry weight in small animals and actual ages for large-bodied animals is feasible and convenient provided care is taken in the handling/drying of the lens.

Treton and Courtois [49], using 16 of the data sets used in the current study, concluded that lens growth took place through two linear growth phases; early rapid growth in a 'lens development stage' (LDS) followed by slow growth in the 'resting lifespan (adult stage)'. The intersection of the extrapolated two linear fits to plots of lens weight against age was taken to be length of the LDS. However, for many of the species studied, insufficient data were available to fit straight lines with any certainty. The present observations indicate that the concept of two growth phases is not applicable to most of species. As discussed earlier, only human lenses and possibly those from the elephant, hippopotamus and woodchuck exhibit biphasic growth.

For most, if not all species, lens growth is independent of gender and unaffected by external influences such as environment and diet [1,7]. Although only three examples were available, it is probable that hibernation also does not affect the rate of lens growth. These and other observations suggest that the lens is genetically programmed to achieve a certain size at a certain rate for each animal and normal internal as well as external influences are unable to alter this, except, perhaps, the abnormal stresses which can lead to cataract formation. The lack of direct connections to the cardiovascular and nervous systems ensures that the lens is isolated from signals which affect the rest of the body. Taken together with the immune response by the host to leaked lens proteins [50], these various observations suggest that the lens may be considered as a separate organism, which relies on the host eye for a stable environment and its miniscule nutritional requirements, but is otherwise independent.

This conclusion appears at odds with the elegant demonstration by McAvoy and others that the various stages of lens morphogenesis in the chick and rat– epithelial cell proliferation, cell migration and differentiation – are dependent on Fibroblast Growth Factor (FGF) and other growth factors present in the vitreous and aqueous humors [2]. It has been demonstrated that retinal factors can alter lens polarity, promote zonule attachment and support growth of lenses implanted in 6 day old mice [51] but less is known of the possible influence of external growth factors on the older lens. An antero-posterior FGF gradient (low-high) is thought to regulate lens growth, suggesting that the slowing of lens growth with increasing age could be attributed to a decrease in the growth factors being delivered by the aqueous. Flattening of morphogen gradients has been invoked to govern other organ shapes and sizes [52,53]. Alternatively, the reduced responses of epithelial cells to FGF with increasing age may be responsible [54]. This does not appear to be the case in humans, elephants, hippopotami and woodchucks since lens growth does not slow with age.

The unique growth of the lens, in which epithelial cells proliferate and differentiate into fiber cells in the equatorial region, provides the necessary building blocks for constructing a refractive index gradient by packing mature fiber cells into the central (nuclear) regions and compacting these through the removal of water. The refractive index gradient thus generated varies with species and presumably is determined by life style [1,3]. However, these processes do not cease when the desired gradient has been established but continue at different rates in different species. They are also continuous in species which do not form a gradient. By contrast, the eye and other ocular structures stop growing very early in life [55].

This raises the question, “Why doesn’t the lens stop growing in adulthood, like the rest of the eye and so many other organs”? There does not appear to be an obvious answer. Perhaps it does not matter: perhaps there are no untoward effects.

Turnover of the epithelium is not required for maintenance of the tissue. Although the cells are capable of undergoing apoptosis and division [56,57], for the most part, the central epithelium is quiescent and the cells are very old. Yet, they continue to function in maintaining lens homeostasis and protection against stress, at least until late in life. Significant mitosis seems to take place only in the equatorial region and leads to fiber cell production. Interestingly, this can be stimulated by increased oxygen levels in mice [58].

The increasing size of the lens is unlikely to create problems in most species since any increase in weight results in much smaller changes in dimensions. In most warmblooded species, lens dry weight has reached 80% of its maximum around the time of sexual maturation when optimum visual function would be required. For the remaining 20%, the dimensions increase by an average of only ~6% over the rest of life. Furthermore, any visual defects arising from growth in the post-reproductive years may not be targets of evolutionary selection pressures.

The foregoing data analyses and discussion have concentrated on the rate of lens growth. More information can be gleaned from a consideration of the actual weights of the lenses. This will be addressed in the following paper.

APPENDIX 1. LENS GROWTH LOGISTIC ANALYSIS AND SPECIES INFORMATION.

To access the data, click or select the words “Appendix 1.”

APPENDIX 2. ASYMPTOTIC DRY WEIGHT/ WET WEIGHT RATIOS AND AVERAGE LENS DENSITIES

To access the data, click or select the words “Appendix 2.”

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REFERENCES

1. Augusteyn RC. Growth of the lens: in vitro observations. Clin Exp Optom 2008; 91:226-39. [PMID: 18331361].
2. Lovicu FJ, McAvoy JW, de Iongh RU. Understanding the role of growth factors in embryonic development: insights from the lens. Philos Trans R Soc Lond B Biol Sci 2011; 366:1204-18. [PMID: 21402581].
3. Pierscionek BK, Regini JW. The gradient index lens of the eye: An opto-biological synchrony. Prog Retin Eye Res 2012; 31:332-49. [PMID: 22465790].
4. Harding JJ, Rixon KC, Marriott FHC. Men have heavier lenses than women of the same age. Exp Eye Res 1977; 25:651- [PMID: 590390].
5. Augusteyn RC. Growth of the human eye lens. Mol Vis 2007; 13:252-7. [PMID: 17356512].
6. Augusteyn RC, Coulson GM, Landman KA. Determining kangaroo age from lens protein content. Aust J Zool 2003; 51:485-94. .
7. Altman PL, Ditmer DS, eds. Growth:including reproduction and morphological development. Washington DC: Fed Amer Soc Exp Biol; 1962.
8. Grzimek B. Grzimek's encyclopedia of mammals. New York: McGraw-Hill; 1990.
9. Augusteyn RC. On the relationship between rabbit age and lens dry weight: improved determination of the age of rabbits in the wild. Mol Vis 2007; 13:2030-4. [PMID: 17982428].
10. Laws RM. Eye lens weight and age in African elephants. African Wildlife. 1968
11. Sikes SK. The African elephant, *Loxodonta africana*: a field method for the estimation of age. J Zool 1966; 150:279-95. .
12. Jawad L. AL-Mamry J,AL-Hassani L, AL-Abri N. The Feasibility of Using Eye Lens Diameter and Weight as an Age Indicator in the Indian Mackerel *Rastrelliger Kanagurta* (Cuvier, 1817) Collected from the Sea of Oman. Water Res Mgment 2012; 2:43-6. .
13. Lord DR. The lens as an indicator of age in cottontail rabbits. J Wildl Manage 1959; 23:358-60. .
14. Rongstad OJ. A cottontail rabbit lens-growth curve from Southern Wisconsin. J Wildlife Mgment 1966; 30:114-21. .
15. Dudzinski ML, Mykytowycz R. The eye lens as an indicator of age in the wild rabbit in Australia. CSIRO Wildl Res 1961; 6:156-9. .
16. Myers K, Gilbert N. Determination of age of wild rabbits in Australia. J Wildl Manage 1968; 32:841-9. .
17. Wheeler SH, King DR. The use of eye-lens weights for aging wild rabbits (*Oryctolagus cuniculus*) in Australia. Aust Wildl Res 1980; 7:79-84. .
18. Rusch DA, Reeder WG. d Rusch DH. Eye lens, testes, and body weight trends in Alberta red squirrels. J Wildl Manage 1982; 46:1010-7. .
19. Zigman S, Yulo T. Eye lens ageing in the dogfish (*mustelus canis*) Comp Biochem Physiol B 1979; 63:379-85. [PMID: 318417].
20. Conrath CL, Gelsleichter J, Musick JA. Age and growth of the smooth dogfish (*Mustelus canis*) in the northwest Atlantic Ocean. Fish Bull 2002; 100:674-82. .
21. Pascal M, Delattre P. Comparaison de differentes methodes de determination de l'age individuel chez le vison (*Mustela vison Shreiber*). Can J Zool 1981; 59:202-11. .
22. Laws RM. Dentition and ageing of the hippopotamus. African Wildlife J. 1978.
23. Vigal CR, Machordom A. Evaluation de la methode de determination de l'age en fonction de la masse du cristallin chez le bouquetin (*Capra pyrenaica Schinz, 1838*) Can J Zool 1988; 66:2836-9. .
24. Nuckle J, Bergeron J-M. Eye lens weight as a criterion for age classification of wild woodchucks. J Wildl Manage 1983; 47:846-52. .
25. Davis DE. Evaluation of character for determining age in woodchucks. J Wildl Manage 1964; 28:9-14. .
26. Hall-Martin AJ. Dentition and age determination of the giraffe (*Giraffa camelopardalis*). J Zool 1976; 180:263-89. .
27. Perry AE, Herreid CF. Comparison of the tooth-wear and lens-weight methods of age determination in the guano bat, *Tadarida Brasiliensis Mexicana*. J Mammal 1969; 50:357-60. .
28. Longhurst WM. Evaluation of the eye lens technique for aging Columbian black-tailed deer. J Wildl Manage 1964; 28:773-84. .
29. Connolly GE, Dudzinski ML, Longhurst W. An improved age-lens weight regression for black-tailed deer and mule deer. J Wildl Manage 1969; 33:701-4. .
30. Hockwin O, Rast-Czyborra F, Schnitzlein W, Muller H-J. Age dependence of wet weight and water content of the beagle dog lens. Ophthalmic Res 1979; 11:136-42. .
31. Carreno NB, Brigada AM, Rosi MI, Castro-Vazquez A. Estimating ages of corn mice (*Calomys musculinus*). J Mammol 1990; 71:468-70. .
32. Adamczewska-Andrzejewska KA. The lens weight as indicator of age of the wild *Microtus arvalis* population. Bull Acad Pol Sci Biol 1973; 21:331-6. .
33. Martinet L. Determination de l'age chez le campagnol des champs (*Microtus arvalis Pallas*) par la pesee du cristallin. Mammalia 1966; 30:425-30. .
34. Janova E, Nesvadbova J, Tkadlec E. Is the eye lens method of age estimation reliable in voles? Folia Zool (Brno) 2007; 56:119-25. .
35. Bauer RD, Johnson AM, Scheffer VB. Eye lens weight and age in the fur seal. J Wildl Manage 1964; 28:374-6. .

36. Dahlgren RB, Twedt CM, Trautman CG. Lens weights of ring-necked pheasants. J Wildl Manage 1965; 29:212-4. .
37. Labisky RF, Mann SH, Lord RD. Weights and growth characteristics of pheasant lenses. J Wildl Manage 1969; 33:270-5. .
38. Bours J, Hockwin O, Fink H. Biochemistry of the ageing rat lens. 1. Lens wet weight and lens dry weight with respect to sex differences. Ophthalmic Res 1983; 15:198-203. [PMID: 6634054].
39. Donaldson HH, King HD. On the growth of the eye in three strains of the Norway rat. Am J Anat 1937; 60:203-29. .
40. Jánová E, Havelková D, Tkadlec E. Does reproduction accelerate the growth of eye lens mass in female voles? Belg J Zool 2007; 137:85-8. .
41. Tanikawa T. An eye-lens weight curve for determining age in black rats, *Rattus rattus*. J Mamm Soc Jap 1993; 18:49-51. .
42. Connolly GE, Dudzinski ML, Longhurst WM. The eye lens as an indicator of age in the black-tailed jack rabbit. J Wildl Manage 1969; 33:159-64. .
43. Pascal M, Damange JP, Douville P, Guedon G. Reserche de criteres d'age chez le campagnol provencal *Pitmys duodecimcostatus* (De Selys-Longchamps, 1839) Mammalia 1988; 52:85-91. .
44. Hagen A, Stenseth NC, Ostbye E, Skar H-J. The eye lens as an age indicator in the root vole. Acta Theriol (Warsz) 1980; 25:39-50. .
45. Takahashi K, Satoh K. Growth of eye lens weight and age estimation in the northern redbacked vole, *Clethryrionomys rutilus*. Mammal Study 1997; 29:9-44. .
46. Ando A, Shiraishi S. Age determination in the Smith's red-backed vole, *Eothenomys smithii*, using optic lens weight. Mammal Study 1997; 22:45-7. .
47. McLeod SR, Druhan JP, Hacker RB. Determining the age of kangaroos using lens weight. Wildl Res 2006; 33:25-8. .
48. Lind OE, Kelber A, Kröger RHH. Multifocal optical systems and pupil dynamics in birds J Exp Biol 2008; 211:2752-8. [PMID: 18723531].
49. Tréton J, Courtois Y. Evidence for a relationship between longevity of mammalian species and a lens growth parameter. Gerontology 1989; 35:88-94. [PMID: 2792789].
50. Luntz MH, Wright R. Lens-induced uveitis. Exp Eye Res 1962; 1:317-23. [PMID: 13931740].
51. Yamamoto Y. Growth of lens and ocular environment: role of neural retina in the growth of mouse lens as revealed by an implantation experiment. Dev Growth Differ 1976; 18:273-8. .
52. Schwank G, Basler K. Regulation of organ growth by morphogen gradients. Cold Spring Harb Perspect Biol 2010; 2:a001669-[PMID: 20182606].
53. Hafen E, Stocker H. How Are the Sizes of cells, organs, and bodies controlled? PLoS Biol 2003; 1:E86-[PMID: 14691557].
54. Richardson NA, McAvoy JW, Chamberlain C. Age of rats affects response of lens epithelial explants to fibroblast growth factor. Exp Eye Res 1992; 55:649-56. [PMID: 1478274].
55. Augusteyn RC, Mohamed A, Nankivil D, Maceo B, Piere F, Parel J-M. Human ocular biometry. Exp Eye Res 2012; 102:70-5. [PMID: 22819768].
56. Ishizaki Y, Voyvodic JT, Burne JF, Raff MC. Control of lens epithelial cell survival. J Cell Biol 1993; 121:899-908. [PMID: 8491781].
57. McAvoy JW. Cell division, cell elongation and distribution of α-, β- and γ-crystallins in the rat lens. J Embryol Exp Morphol 1978; 44:149-65. [PMID: 650132].
58. Shui Y-B, Beebe DC. Age-Dependent Control of Lens Growth by Hypoxia. Invest Ophthalmol Vis Sci 2008; 49:1023-9. [PMID: 18326726].
59. Malinow MR, Corcoran A. Growth of the lens in howler monkey, (*Alouatta caraya*). J Mammal 1966; 47:58-63. [PMID: 4955606].
60. Novakowski NS. Cemental deposition as an age criterion in bison and the relation of incisor wear, eye lens weight and dressed bison carcass weight to age. Can J Zool 1965; 43:173-8. [PMID: 14287036].
61. Pierscionek BK, Augusteyn RC. Growth related changes in functional parameters in the bovine lens. Biochim Biophys Acta 1992; 1116:283-90. [PMID: 1610885].
62. Hockwin O, Schmutte J, Muller HK. Untersuchungen uber gewicht und volumen verschieden alter rinderlinsen. Graefes. 1978.
63. Hubbert WT, Hughes DE, Stalheim HV, Booth GD. Weight changes of rhommbencephalon and eye lens in the developing bovine foetus. Am J Vet Res 1974; 35:769-72. [PMID: 4836151].
64. Smuts GL. Age determination in Burchell's zebra (*Equus burchelli antiquorum*) from the Kruger National Park. J S Afr Wildlife Mgmt Assn 1974; 4:103-16. .
65. Attwell CAM. Age determination of the blue wildebeest, *Connochaetes taurus* in Zululand. S Afr J Zool 1980; 15:121-30. .
66. Augusteyn RC, Cake MA. Post mortem uptake of water by sheep lenses left in the eye. Mol Vis 2005; 11:749-51. [PMID: 16179906].
67. Ho L, Field RA, Russell WC, Riley ML, Ercanbrack SK, Williams FL. Influence of gender, breed and age on maturity characteristics of sheep. J Anim Sci 1989; 67:2460-70. [PMID: 2599986].
68. Klein F, Breton D, Brandt S, Gaillard J-M. Suitability of wild boar (*Sus scrofa*) aging techniques by eye lens weight and body weight. Gibier Faune Sauvage 1990; 7:39-51. .
69. Matchske GH. An eye-lens nutrition study of penned European wild hogs. Proc. 17th Ann Conf. SE Assoc Game Fish Comm 1963; 20–27.

70. Shafiee A, McIntire GL, Sidebotham LC, Ward KW. Experimental determination and allometric prediction of vitreous volume, and retina and lens weights in Göttingen minipigs. Vet Ophthalmol 2008; 11:193-6. [PMID: 18435662].
71. Henzell R. Eye-lens weight as an indicator of age in Australian goats, *Capra-Hircus L*. Wildl Res 1987; 14:69-79. .
72. Kolenosky GB, Miller SB. Growth of the lens of the proghorn antelope. J Wildl Manage 1962; 26:112-3. .
73. Simpson CD, Elder WH. Lens weight related to estimated age in greater kudu. J Wildlife Mgment 1968; 32:764-8. .
74. Fairall N. Growth and age determination in the hyrax, *Procavia capensis*. S Afr J Zool 1980; 15:6-21. .
75. Fairall N. The use of the eye lens technique in deriving the age structure and life table of an impala (*Aepyceros melampus*) population. Koedoe 1969; 2:90-6. .
76. Nellis CH. Lens weights of mule deer fetuses. J Wildl Manage 1966; 30:417-9. .
77. Maringgele FJ. Altersbestimmung beim Reh (*Capreolus capreolus L*) und beim Rothirsch (*Cervus elaphus L*) mit Hilfe der Trockengewichtsbestimmung der Augenlinse. Jagdwiss. 1978.
78. Feldhamer GA, Chapman JA. Evaluation of the eye lens method for age determination in Sika deer. Acta Theriol (Warsz) 1980; 25:239-44. .
79. Rautenbach IL. Ageing criteria in the springbok (*Antidorcas marsupialis*). AnnTransvaal Museum 1971; 27:83-133. .
80. Mason DR. Dentition and age determination of the warthog, *Phacochoerus aethiopicus* in Zululand South Africa. Koedoe 1984; 27:79-119. .
81. Child G, Sowls LK, Richardson GL. Uses and limitations of eye-lens weight for ageing wart hog. Arnoldia 1965; 39:1-23. .
82. Webb JW, Nellis DW. Reproduction and age estimation in St Croix white-tailed deer. Tex J Sci 1981; 33:184-96. .
83. Lombaard LJ. Age determination and growth curves in the black-backed jackal, *Canis mesomelas Schreber, 1775* (Carnivora: Canidae) Ann Transvaal Museum 1971; 27:135-69. .
84. Ely LO. Metabolism of the crystalline lens. I. Water content and growth rate. Am J Ophthalmol 1949; 32:215-9. [PMID: 18132889].
85. Catling PC, Corbett LK, Wetcott M. Age determination in the dingo and crossbreeds. Wildl Res 1991; 18:75-83. .
86. Lord RD. The lens as an indicator of age in the gray fox. J Mammol 1961; 42:109-11. .
87. Wigal RA, Chapman JA. Age determination, reproduction, and mortality of the Gray fox (*Urocyon cinereogenteus*) in Maryland, USA. Z Saugetierkd 1983; 48:226-45. .
88. Sanderson GC. The lens as an indicator of age in raccoons. Am Midl Nat 1961; 65:481-5. .
89. Cavallini P, Santini S. Age determination in the red fox in a Mediteranean habitat. Z Saugetierkd 1995; 60:136-42. .
90. Rausch RA, Pearson AM. Notes on the wolverine in Alaska and the Yukon territory. J Wildl Manage 1972; 36:249-60. .
91. Hearn BJ, Mercer WE. Eye-lens weight as an indicator of age in Newfoundland arctic hares. Wildl Soc Bull 1988; 16:426-9. .
92. Tiemeier OW, Plenert ML. A comparison of three methods for determining the age of black-tailed jackrabbits. J Mammol 1964; 45:409-16. .
93. Andersen J, Jensen B. The weight of the eye lens in the European hares of known age. Acta Ther 1972; 17:87-92. .
94. Suchentrunk F, Willing R, Hartl GB. On eye lens weights and other age criteria of the brown hare (*lepus europaeus, Pallas 1778*). Z Saugetierkd 1991; 56:365-74. .
95. Broekhuizen S, Maaskamp F. Age determination in the European hare (*Lepus europaeus Pallas*) in the Netherlands. Z Saugetierkd 1979; 44:162-75. .
96. Ando A, Yamada F, Taniguchi A, Shiraishi S. Age determination by the eye lens weight in the Japanese hare, *Lepus brachyurus brachyurus* and its application to two local populations. Sci Bull Fac Agr Kyushu Univ 1992; 3–4:169-75. .
97. Kauhala K, Soveri T. An evaluation of methods for distinguishing between juvenile and adult mountain hares *Lepus timidus*. Wildl Biol 2001; 7:295-300. .
98. Keith LB, Meslow EC, Rongstad OJ. Techniques for snowshoe hare population studies. J Wildlife Mgment 1968; 32:801-12. .
99. Keith LB, Cary JR. Eye lens weights from free-living adult snowshoe hares of known age. J Wildl Manage 1979; 43:965-9. .
100. Malcolm JR, Brook RJ. Eye lens weight and body size as criteria of age in beaver (*Castor canadensis*). Can J Zool 1981; 59:1189-92. .
101. Leege TA, Williams RM. Beaver Productivity in Idaho. J Wildl Manage 1967; 31:326-32. .
102. Van Aarde RJ. Age determination of cape porcupines, *hystrix-africaeaustralis*. S Afr J Zool 1985; 20:232-5. .
103. Barker JM, Boonstra R, Schulte-Hostedde AI. Age determination in yellow-pine chipmunks (*Tamias amoenus*); a comparison of eye lens masses and bone sections. Can J Zool 2003; 81:1774-9. .
104. Beale DM. Growth of the eye lens in relation to age in fox squirrels. J Wildl Manage 1962; 26:208-11. .
105. Fisher EW, Perry AE. Estimating ages of gray squirrels by lens-weights. J Wildl Manage 1970; 34:825-8. .
106. Liu J, Wang T, Li J, Shao M. Studies on the population age structure of ground squirrel. Acta Theriol Sinica 1993; 13:277-82. .
107. Le Louarn H. Determination de l'age par la passee des crystallins chez quelques especes de rongeurs. Mammalia 1971; 35:636-43. .

108. Stockrahm DMB, Dickerson BJ, Adolf SL, Seabloom RW. Ageing black-tailed prairie dogs by weight of eye lens. J Mammal 1996; 77:874-81. .
109. Hockwin O, Bechtel-Ehrig U, Licht W, Noll E, Rast E. Concerning the estimation of the age in guinea pigs, rabbits and chickens by means of determining their lens weight. Ophthalmic Res 1971; 2:77-85. .
110. Birney EC, Jeness R, Baird DD. Eye lens proteins as criteria of age in cotton rats. J Wildl Manage 1975; 39:718-28. .
111. Teska WR, Pinder JE. Effects of nutrition on age determination using eye lens weights. Growth 1986; 50:362-70. [PMID: 3803993].
112. Dische Z, Borenfreund E, Zelmenis G. Changes in lens proteins of rats during ageing. AMA Arch Opthalmol 1956; 55:471-83. .
113. Hardy AR, Quy RJ, Huson LW. Estimation of age in the Norway rat (*Rattus norvegicus berkenhout*) from the weight of the eye lens. J Appl Ecol 1983; 20:97-102. .
114. Myers K, Carstairs J, Gilbert N. Determination of age of indigenous rats in Australia. J Wildl Manage 1977; 41:322-6. .
115. Williams JM. Determination of age of Polynesian rats (*Rattus exulans*). Proc NZ Ecol Soc 1976; 23:79–82.
116. Vejaratpimol R, Liat LB. Age estimation of *Rattus argentiventer* from eye lens weight. J Sci Soc Thailand 1983; 9:107-18. .
117. Poulet AR. Determination de l'age par la pesee des crystallins chez cinq especes de rongeurs Murodes at Gerbillides de l'ouest de l'Afrique. Mammalia 1980; 44:381-98. .
118. Lalis A, Lecompte E, Cornette R, Moulin S, Machangu RS, Makundi R, Aniskine VM, Denys C. Polymorphism of the age population structure of two wild *Mastomys natalensis* (Rodentia: Muridae) Tanzanian habitat samples: a multicriteria comparison. Mammalia 2006; 70:293-9. .
119. Gosling LM, Huson LW, Addison GC. Age estimation of coypus (*Myocastor coypus*) from eye lens weight. J Appl Ecol 1980; 17:641-7. .
120. Le Boulenge E. Two ageing methods for muskrats: live or dead animals. Acta Ther 1977; 22:509-20. .
121. Augusteyn RC. The effect of light deprivation on the mouse lens. Exp Eye Res 1998; 66:669-74. [PMID: 9628812].
122. Zhou G, Williams RW. Mouse models for the analysis of myopia: An analysis of variation in eye size of adult mice. Optom Vis Sci 1999; 76:408-18. [PMID: 10416936].
123. Rowe FP, Bradfield A, Quy RJ, Swinney T. Relationship between eye lens weight and age in the wild house mouse (*Mus musculus*). J Appl Ecol 1985; 22:55-61. .
124. Sutherland DR, Banks PB, Jacob J, Singleton GR. Shifting age structure of house mice during a population outbreak. Wildl Res 2004; 31:613-8. .
125. Nabaglo L, Pachinger K. Eye lens weights as an age indicator in yellow-necked mice. Acta Theriol (Warsz) 1979; 24:118-22. .
126. Steiner M. Untersuchungen uber die variabilitat und bionomie der gattung *Apodemus* (Muridae, Mammalia) der Donau-Auen von stickerau (Niederosterreich). Z Wiss Zool 1968; 177:91-6. .
127. Adamczewska-Andrzejewska K. Growth, variations and age criteria in *Apodemus agrarius Pallas 1771*). Acta Theriol (Warsz) 1973; 18:353-94. .
128. Quere JP, Vincent JP. Determination d l'age chez le mulot gris (*Apodemus sylvaticus L., 1758*) par la pesee des crystallins. Mammalia 1989; 53:287-93. .
129. Vandorpe E, Verhagen R. An age reference model for the wood mouse, *Apodemus sylvaticus*, by use of the lens technique. Ann Soc R Zool Belg 1979; 109:133-40. .
130. Kravetz FO, Busch M, Percich RE, Manjon MC, Marconi PN. Ecologia de *Calomys laucha* (Rodentia, Cricetidae), en el departamento de Rio Cuarto (Cordoba). II Criterios para la determinacion de edades y crecimiento. Ecologia 1981; 6:35-44. .
131. Millar JS, Iverson SL. Weight of the eye lens as an indicator of age in *Peromyscus*. Naturalist. 1978.
132. Rodel R. Alter und linsengewicht von Meriones *shawi grandis* (Cabrera, 1907) Z Saugetierkd 1986; 52:291-4. .
133. Jain AP, Prakash I. Eye-lens as an indicator of age in the Indian Gerbil, *Tatera indica*. Growth 1981; 45:119-22. [PMID: 7286774].
134. Thomas RE, Bellis ED. An eye-lens weight curve for determining age in *Microtus pennsylvanicus*. J Mammol 1980; 61:561-3. .
135. Gourley RS, Jannett FJ. Pine and Montane vole age estimates from eye lens weights. J Wildl Manage 1975; 39:550-6. .
136. Kozakiewicz M. The weight of the eye lens as the proposed age indicator of the bank vole. Acta Theriol (Warsz) 1976; 21:314-6. .
137. Askaner T, Hansson L. The eye lens as an age indicator in small rodents. Oikos 1967; 18:151-3. .
138. Ostbye E, Semb-Johansson A. The eye lens as an indicator of age in the Norwegian Lemming. Nytt Mag Zool 1970; 18:239-43. .
139. Erlinge S, Danell K, Frodin P, Hasselquist D, Nilsson P, Ollofsson E-B, Svensson M. Asynchronous population dynamics of Siberian lemmings across the Palaertic tundra. Oecologia 1999; 119:493-500. .
140. Mallory FF, Elliott JR, Brooks RJ. Changes in body size in fluctuating populations of the collared lemming: age and photoperiod influences. Can J Zool 1981; 59:174-82. .
141. Augusteyn RC. Lens protein changes during kangaroo development. Mol Vis 2011; 17:3234-42. [PMID: 22194649].
142. Kingsmill E. An investigation of criteria for estimating age in the marsupials, *trichosurus vulpecula Kerr* and *Perameles nasuta Geoffrey*. Aust J Zool 1962; 10:597-616. .
143. Roseberry JL, Verts BJ. Relationships between lens-weight, sex and age in bobwhites. Trans Illinois Acad Sci 1963; 58:208-12. .

144. Hockwin O, Fink H, Noll E, Licht W. Untersuchungen zum stoffwechsel von hahnchenlinsen wahrend des ersten lebensjahres. Doc Ophthalmol 1966; 20:73-90. [PMID: 5982293].

145. Fink H, Hockwin O, Weigelin E. The lens weight of cockerels during the first 12 months. Acta Genetica Basel 1969; 18:180-91. .

146. Campbell H, Tomlinson RE. Lens weight in chukar partridges. J Wildlife Mgmnt 1962; 26:407-9. .

147. Payne RB. Growth rate of the lens of the eye of the house sparrows. Condor 1960; 63:338-40. .

148. Henny CJ, Ludke LJ. An attempt to age mallards using eye lens proteins. J Wildl Manage 1974; 38:138-41. .

149. Brown NS. Eye lens weights as indicators of age in rock doves. J Wildl Manage 1970; 34:656-7. .

150. Aubib A, Dunn EH, MacInnis CD. Growth of lesser snow geese on arctic breeding grounds. Condor 1986; 88:365-70. .

151. Bruggers RL, Jackson WB. Eye-lens weight of the bullfrog (*Rana catesbeiana*) related to larval development, transformation, and age of adults. Ohio J Sci 1974; 74:282-6. .

152. Carlton WG, Jackson WB. The eye lens as an age indicator in carp. Copeia 1968; 3:633-6. .

153. Crivelli A. The eye lens weight in the common carp, *Cyprinus carpio L.* J Fish Biol 1980; 16:469-73. .

154. Siezen RJ. Eye lens aging in the spiny dogfish (*Squalus acanthias*) 1. Age determination from lens weight. Curr Eye Res 1989; 8:707-12. [PMID: 2791619].

155. Burkett RD, Jackson WB. The eye lens as an indicator of age on freshwater drum. Am Midl Nat 1971; 85:222-5. .

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